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Impact of Cadmium Contaminated Growing Medium on the Growth and Physiological Responses of Tomato Seedlings (*Solanum lycopersicum* L.)

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Globally, urban areas are facing a rising issue of industrial pollution particularly due to heavy metals like Cadmium (Cd), which pose a threat to both plants and humans. However, plants response to Cd exposure varies based on factors like species, genetics, and growing conditions such as the type of soil and cultivation methods. This study focused on young tomato seedlings subjected to different Cd concentrations (0, 100, 200, and 300 ppm) through a pot experiment, aiming to understand the impact of Cd on various morpho-physiological traits of the "MAYAR F1" tomato cultivar. The results exhibited amazing findings that the chlorophyll contents (21.93 nmol/mg), relative water contents (0.84%), electrolyte leakage (135.5 uS/cm) and leaf area (6.37 cm2) were highest at 300 ppm followed by control. While the maximum number of leaves (11) were noticed in control. Consequently, the study suggests that cultivating tomatoes in urban agriculture soils contaminated with Cd could be a viable option. Nonetheless, it emphasizes the importance of exercising caution and conducting further research to fully comprehend the potential long-term implications and associated risks of Cd exposure in urban agriculture.
Keywords: Heavy metal, cadmium; morphological traits, plant physiology, chlorophyll contents, electrolyte leakage.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to the family "Solanaceae" is an important vegetable crop that serves as a model plant for various biological research studies concerning plants (Wang *et al.,* 2023). Globally, it is primarily grown for its edible fruits, which are utilized in their raw form for salads, cooking, as food additives, and processed into products like ketchup, sauces, paste and soups (Britannica, 2023). It is rich in antioxidants, vitamins, proteins, minerals, fiber, amino acids, carotenoids, lycopene, fatty acids and other essential nutrients that prevent constipation, cardiovascular diseases and maintain lipid profile (Ali *et al.,* 2020). Moreover, it detoxifies the human body and prevents it from cancer and several chronic diseases (Campestrini *et al.,* 2019; Vats *et al.,* 2020).

It is 2nd largest vegetable crop followed by potatoes and worldwide, it is cultivated on approximately 5.16 million hectares of land, with an annual global production of about 189 million tonnes (FOSTAT, 2021). In Oman, it is grown on an area of 3232 ha having 283274 tonnes of annual production, however, its yield and area of production have declined due to disturbance in a natural ecosystem by human interference (FOSTAT, 2021; Kogo *et al.,* 2021). The rise in global population rate is contributing to accelerated industrialization, resulting in a greater production of industrial waste. These industrial wastes contain a substantial number of heavy metals such as zinc (Zn), boron (B), and cadmium (Cd), etc, that are toxic to the environment and human health. Heavy metals are typically defined as metals with high atomic weights and densities greater than 5 grams per cubic centimeter (g/cm^3) (Zhang *et al.,* 2019). Even in developed countries, there are no proper remedies to dispose of this industrial waste, therefore industrial wastes are causing soil pollution and are particularly affecting agricultural commodities such as fruits and vegetables that are consumed fresh and have hazardous effects on human health (Palansooriya *et al.,* 2020; Gebeyehu *et al.,* 2020).

There are certain elements such as iron and zinc are required in humans and plants for growth and development. However, their consumption above the desired level is toxic and leads to several chronic diseases (Alengebawy *et al.,* 2021). In plants, heavy metal toxicity limits nutrient uptake, disturbance in photosystem and nitrogen metabolism,

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denaturation of chloroplast and cell membrane structure (Badawy *et al.*, 2022). These heavy metals also damage cellular organelles and components such as mitochondria, lysosomes, DNA, proteins, carbohydrates and nuclei, which may lead to structural changes within cells, which in turn may result in variations in the cell cycle or apoptosis (Beyersmann and Hartwig, 2008).

Among the heavy metals Cd is one of the highly toxic metals to the plants. It has been reported that there are many vegetable crops affected by heavy metal, especially Cadmium (Cd) (*Singh et al.*, 2022; Quispe *et al.*, 2021). When the Cd is excess in the soil it is transported into the plant cell causing oxidative stress. This oxidative stress causes changes at the cellular, molecular, morphological, biochemical and genetic level, in response to this stress compromises on growth and development, which leads to a reduction in quality as well as quantity and ultimately there will be a decline in yield (Rizwan *et al.*, 2017). In addition to the oxidative stress, it also reduces the capability of roots to uptake the nutrients and their translocation to the plant tissues. So, the main aim of this study is also to investigate the impact of cadmium nitrate tetrahydrate on tomato growth and the impact of its toxicity (as abiotic stress) on tomato plants.

MATERIALS AND METHODS

***Planting Material*:** This research was conducted at the greenhouse located at the Tissue Culture Centre in Bahla, situated at 23.59° N latitude and 58.18° E longitude. However, the analysis of morphological and physiological parameters was conducted at the Department of Plant Science, Sultan Qaboos University, College of Agricultural and Marine Sciences. To assess the impact of Cadmium (Cd) on tomatoes, the "MAYAR F1" hybrid variety was chosen. The seeds were planted in a growing medium consisting of cocopeat, farmyard manure, and field soil in a ratio of 1:3:4, respectively. Prior to planting, the growing medium was sterilized at 121 °C for 15 minutes to prevent fungal and bacterial diseases. Additionally, the tomato seeds were treated with a 1% KOH disinfectant solution for 1 minute and rinsed with double-distilled water to prevent infections.

Plastic pots measuring 18 cm × 12 cm (length × width) were used, with three seeds planted in individual pots. These pots were placed in the greenhouse and irrigated by misting every hour for 10 seconds. This practice continued for two weeks until the seeds germinated completely. After this period, Cadmium nitrate tetrahydrate ($CdN2O6{\cdot}4H2O$) was applied through manual irrigation at concentrations of 0, 100, 200, and 300 ppm.

***Data collection*:** The data was collected up to the final maturity of the crop, and different physiological and morphological attributes were studied. The details and procedures of each parameter have been discussed below.

***Chlorophyll contents (nmol mg)*:** Chlorophyll levels in tomato plants were evaluated using a SPAD meter. Three mature leaves were chosen from each plant for the measurements. The leaves were placed beneath the sampling head, pressure was applied to the head onto the leaf, and the reading displayed on the screen was recorded to obtain accurate measurements.

***Electrolyte leakage (µS/cm)*:** Electrolyte leakage was measured using three randomly selected mature leaves. The leaves were sliced and immersed in deionized water. The initial reading was taken by placing the EC meter probe into the water immediately after submerging the leaf slices. Subsequent readings were recorded at 15-minute intervals, and the difference between these readings was used to quantify the extent of electrolyte leakage from the leaves.

***Relative water contents (RWC)*:** To determine the relative water content, small plant twigs were chosen. A solution of 50 ml Sarstedt was poured into tubes containing 5-7 ml of tap water. The leaves were detached at the base of the petiole and placed in the Sarstedt tube in such a way that the petiole was fully submerged in the water within the tube. The leaves were left submerged for 180 minutes. After this period, the leaves were removed, and their turgid weight was measured. Subsequently, the leaves were placed in an oven for two days to obtain their dry weight. After two days, the weight was measured again, and the Relative Water Content (RWC) was calculated using the following equation:

$RWC = (TW-DW)/(FW-DW)$

Where FW represents the fresh weight of the leaf, DW is the dry weight of the leaf, and TW is the turgor weight of the leaf.

***Leaf area (cm^2)*:** A laser leaf area meter was employed to measure the leaf area. The process included cutting leaves from the plants and arranging them on a palette where they were flattened. A laser scanner was then passed over the leaves, measuring both their width and length, and subsequently calculating the total area of the leaf.

***Plant Biomass*:** In this method, plants from each treatment were gathered, and their fresh weight was measured using a weighing balance. After recording the fresh weight for each treatment, the plants were dried in an oven at 72 °C for 24 hours. The plant biomass was then calculated using the following equation:

Plant biomass = Fresh weight − Dry weight

Stem thickness (cm), shoot length (cm), and root length (cm)

***Number of leaves*:** Stem thickness was measured using a vernier caliper, with the unit of measurement expressed in centimeters (cm). Meanwhile, shoot length (cm) and root length (cm) were measured using a measuring tape. Additionally, the number of leaves on each individual plant was counted manually.

***Statistical analysis*:** In each treatment, a total of nine pots were utilized, with three pots considered as a single replicate for each treatment. The experiment was conducted in greenhouse control conditions. Therefore, the experiment was

laid out in Completely Randomized Block Design (CRD) and statistical comparisons of means were performed at a significance level of 5% using the Least Significant Difference (LSD) method.

RESULTS AND DISCUSSION

***Chlorophyll contents (nmol/mg)*:** The impact of Cd exposure on chlorophyll contents is illustrated in Figure 1. The results indicated that cadmium nitrate trihydrate had a non-significant impact on plant chlorophyll contents. The highest chlorophyll contents (21.93 nmol/mg) were observed at a concentration of 300 ppm, followed by 100 ppm (20.88 nmol/mg). In contrast, the control group exhibited the lowest chlorophyll contents (19.93 nmol/mg). The influence of metal exposure, particularly cadmium (Cd), on plant chlorophyll levels and overall plant health can be considerable and harmful. Cadmium, being a heavy metal, is known to be toxic to plants, leading to various detrimental effects. However, the impact can vary based on experimental conditions, plant species, and environmental variables.

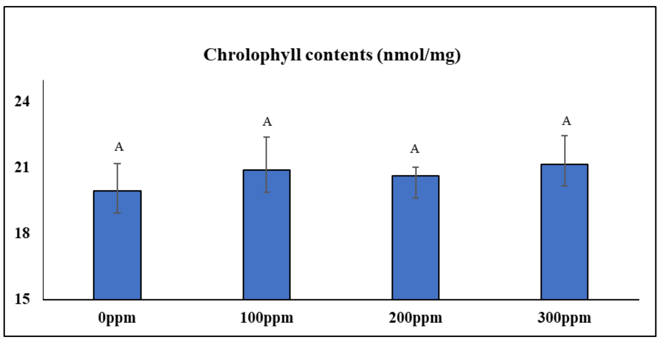

Figure 1. Impact of cadmium stress on the tomato chlorophyll contents.

It is noteworthy that the findings in this study contradict those of Oancea *et al.* (2005), who observed a decrease in chlorophyll content as Cd concentration increased. Similarly, Saeed *et al.* (2013) also reported a reduction in chlorophyll content in tomato leaves with increasing Cd concentration. The variation or disparity in these results might be attributed to differences in the threshold levels of plants, which can vary depending on their specific environmental conditions.
Cd tolerance in tomatoes, is also associated with using Cd nitrate, which underscores the complex interplay between plants and heavy metal stress (Raza *et al*., 2020). Cd tolerance in certain tomato cultivars could lie in genetic variability (Sharma and Dietz, 2009). It's plausible that specific cultivars have evolved mechanisms to sequester, detoxify, or exclude Cd more efficiently than others. Additionally, the developmental stage of the plant and the timing of Cd exposure might influence its tolerance level (Haider *et al.*, 2021). Furthermore, the choice of Cd source, such as Cd nitrate, introduces limitations due to its chemical properties and interactions with soil components. Cd nitrate, being a water-soluble compound, might exhibit different behavior in soil compared to other Cd forms, altering its availability to plants and subsequent toxicity levels**.**

***Relative water contents (%)*:** The results revealed a significant effect of cadmium nitrate trihydrate on the relative water contents of tomatoes, as shown in Figure 2. The findings indicated a decrease in relative water contents in tomatoes with the application of Cd. The highest relative water contents (0.84%) were observed in untreated plants, followed by 300 ppm (0.67%). Conversely, the lowest relative water contents (0.53%) were observed at a concentration of 100 ppm. In our study, an inverse relationship was observed between relative water contents and Cd concentration. An increase in Cd concentration led to a decrease in relative water contents, while a decrease in Cd concentration resulted in an increase in relative water contents. The presence of Cd-induced stress caused a reduction in osmotic potential, ultimately leading to a decrease in relative water contents. Similar findings were reported by Liu *et al.* (2021), who observed a decrease in relative water contents in sativa seedlings subjected to Cd-induced stress. Additionally, Vijayaragavan *et al.* (2011) noted the inhibitory impact of Cd on cowpea (*Vigna unguiculata* L.), reducing the water absorption necessary for seed embryo development.

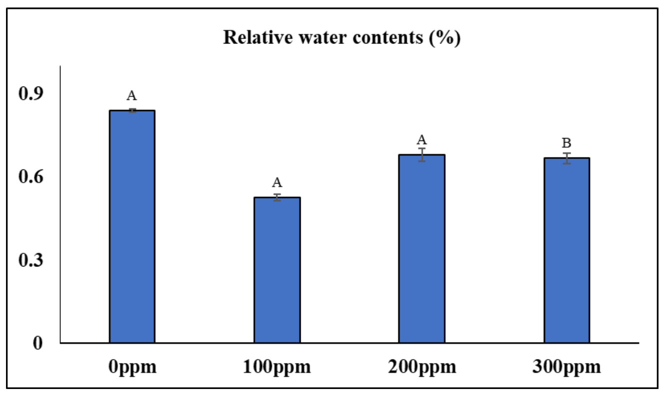

Figure 2. Impact of cadmium stress on the relative water contents of tomato.

***Electrolyte leakage (uS/cm)*:** Electrolyte leakage is an indicator of cellular membrane integrity and stress responses. When plant cells experience stress, such as from environmental factors like drought, salinity, extreme temperatures, or pathogen attack, their cellular membranes can become damaged. Higher levels of electrolyte leakage indicate greater membrane damage, which can compromise the normal functioning of cells. The results illustrated a significant effect of cadmium nitrate trihydrate on tomato fruit electrolyte leakage, as shown in Figure 3. The study found that the highest electrolyte leakage (135.5 uS/cm) was

observed at a concentration of 300 ppm, followed by the control group (104.83 uS/cm). Conversely, the lowest electrolyte leakage was noted at 200 ppm (55.51 uS/cm).

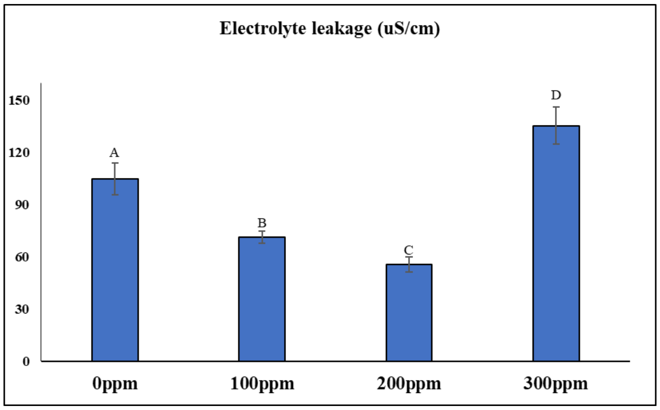

Figure 3. Impact of cadmium stress on electrolyte leakage of tomato.

In this research, the application of Cd induced stress on tomato plants, leading to increased electrolyte leakage. This phenomenon is common when plants are stressed, causing them to release more ions. Typically, increase in plant leakage occurs in response to various environmental factors, including salinity, drought, extreme temperatures, and physical damage (Hussain *et al.*, 2022). These findings align with those of Siddiqui *et al*. (2019), who observed a significant impact on tomato plants, resulting in increased chlorophyll degradation and electrolyte leakage due to salt stress.

***Leaf area (cm^2)*:** The study results indicated a non-significant impact of cadmium nitrate trihydrate application on tomato leaf area, as depicted in Figure 4.

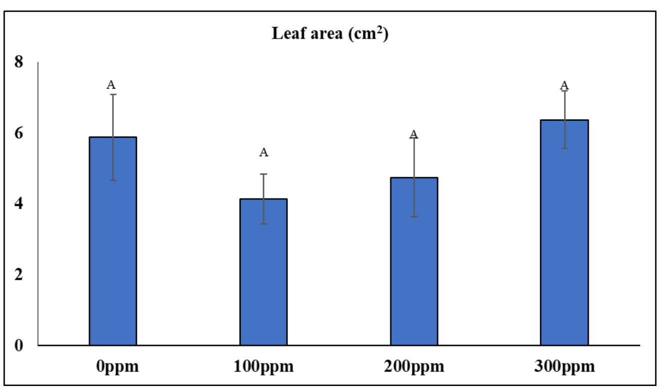

Figure 4. Impact of cadmium stress on the leaf area of tomato.

The findings showed that the maximum leaf area (6.37 cm2) was observed at a concentration of 300 ppm, followed by the control group (5.88 cm2). Conversely, the smallest leaf area (4.14 cm2) was noted at 100 ppm. Generally, exposure to Cd leads to a reduction in leaf area and interferes with cell growth by affecting cell wall formation and turgor pressure. However, certain plant species can tolerate Cd up to a certain limit. In our study, tomato plants exhibited tolerance to Cd stress, with non-significant results indicating a minimal impact. These results align with the findings of Chaves *et al.* (2011), who reported that Cd stress did not significantly affect tomato leaf area.

***Plant biomass (g/cm^2)*:** Figure 5 demonstrates the significant impact of Cd concentration on plant biomass. The results revealed that plant biomass was highest (1.53 g/cm2) in the control group, followed by Cd concentration at 300 ppm (1.48 g/cm2). However, plant biomass decreased to 1.07 g/cm2 at 200 ppm, and the minimum plant biomass (0.29 g/cm2) was observed at 100 ppm. There exists a positive correlation between biomass and the photosynthetic rate of plants; an increase in photosynthesis usually leads to a rise in plant biomass. However, under stressful conditions, biomass tends to decrease. Interestingly, in our study, Cd stress had a significant impact on tomato plant biomass, decreasing at lower Cd concentrations while increasing at the highest concentration. This effect might be attributed to the genetic behavior of the selected genotype. These findings are consistent with those of Hussain *et al.* (2023), who observed varying responses of different tomato cultivars under Cd stress. However, our results contradict those of Manivasagaperumal *et al*. (2011), who noted a reduction in tomato plant biomass with increasing Cd concentrations.

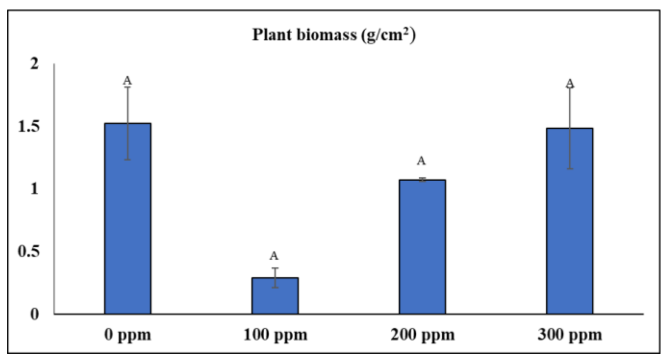

Figure 5. Impact of cadmium stress on tomato plant biomass.

***Stem thickness (cm)*:** The study results revealed a non-significant effect of cadmium nitrate trihydrate application on tomato plant stem thickness, as shown in Figure 6. Stem thickness ranged from 2.71 to 3.50 cm. The highest stem thickness (3.50 cm) was observed in the control group, followed by 3.32 cm at 100 ppm. The smallest increase in stem thickness was noted at 300 ppm (2.70 cm). Although tomato plants exhibit varying degrees of cadmium tolerance, excessive cadmium exposure can still negatively impact plant growth and development. However, in our findings, stem

thickness was not significantly affected by Cd. Similar results were reported by Carvalho *et al.* (2018), who found that the stem thickness of tomatoes was not influenced by Cd exposure.

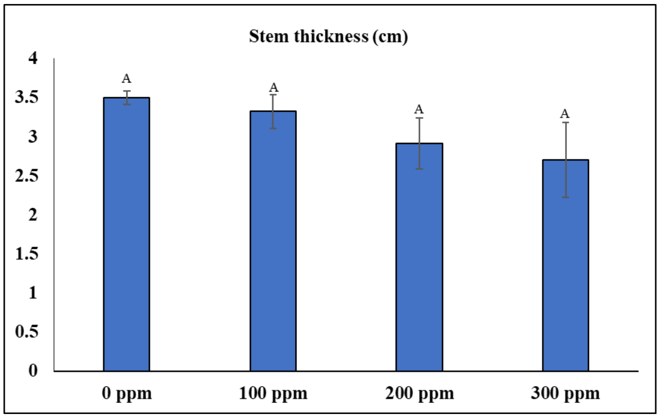

Figure 6. Impact of cadmium stress on stem thickness of tomato.

***Shoot length (cm)*: ** The data regarding shoot length is presented in Figure 7. The results indicated that the application of Cd did not have a significant impact on shoot length. In this study, the highest shoot length (13.13 cm) was observed at 100 ppm Cd concentration, followed by 12.60 cm at 200 ppm. The lowest shoot length was recorded in the control group (12.19 cm). Morphological parameters of plants such as stem thickness, shoot length, and root length are typically highly correlated. However, Cd exhibited a non-significant effect on shoot length in our study. These results contrast with findings from other researchers who observed reduced shoot length when Cd was applied to tomatoes (Rahmatizadeh *et al.*, 2019).

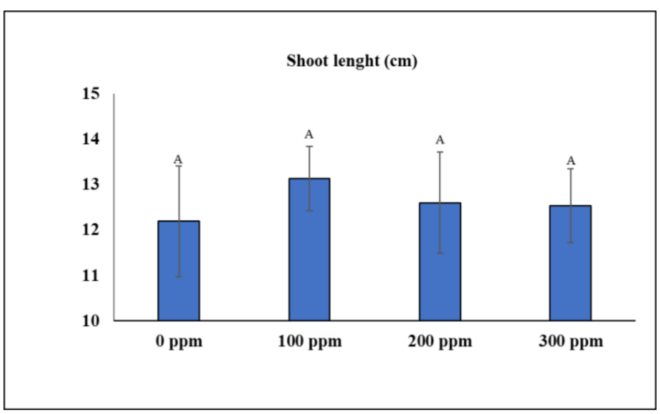

Figure 7. Impact of cadmium stress on stem length of tomato.

***Root length (cm)*: ** The data concerning plant root length is depicted in Figure 8. The results indicated that the application of Cd did not have a significant impact on plant root length. In this study, the maximum root length (7.83 cm) was observed at 200 ppm, followed by 7.71 cm at 100 ppm. The lowest root length (6.64 cm) was recorded in the control group. Root length can vary based on factors such as the concentration of Cd used, the plant species involved, and the rooting media (Qadri *et al.*, 2021). It is possible that the specific combination of potting media used in our study reduced the uptake of Cd, consequently not adversely affecting the root length of tomatoes.

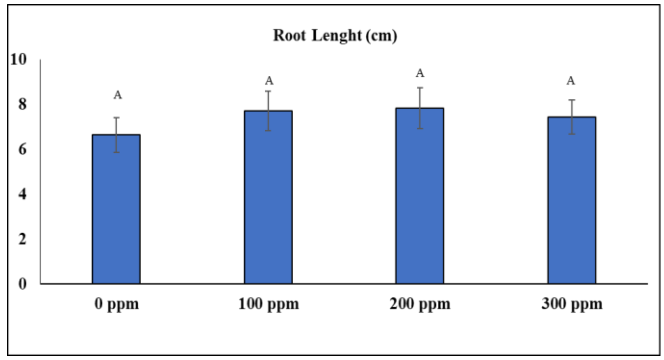

Figure 8. Impact of cadmium stress on root length of tomato.

***Number of leaves*: ** The results indicated a significant effect of Cd applications on the number of leaves, as illustrated in Figure 9. The findings revealed that the highest number of leaves (11) was observed in the control group, followed by 9.33 leaves at 100 ppm. Conversely, the minimum number of leaves (8.33) was observed at 200 ppm Cd concentration. In our study, a negative relationship was observed between the cadmium dose and the number of leaves. Similar findings have been observed by other researchers who also noticed a decrease in the number of leaves with an increase in Cd concentration (Rehman *et al.*, 2011). Likewise, Imran *et al.* (2007) reported that both Cd and Al had a negative impact on the growth of soybean, especially at higher doses.

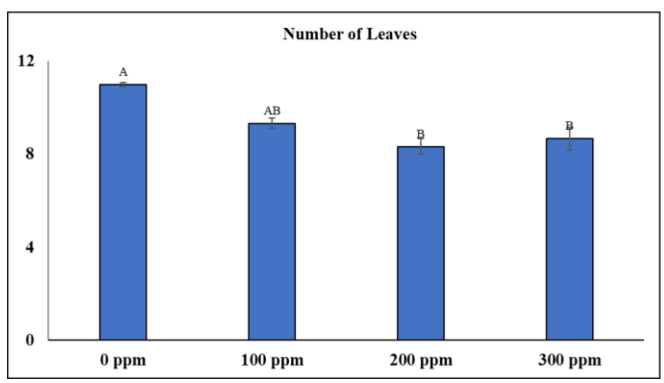

Figure 9. Impact of cadmium stress on the number of leaves of tomato.

Conclusion**:** This study investigated the impact of Cadmium (Cd) contamination on young tomato seedlings of the "MAYAR F1" cultivar in a pot experiment. The intriguing findings revealed that at the highest tested Cd concentration (300 ppm), tomato seedlings exhibited elevated levels of chlorophyll content, relative water content, electrolyte leakage, and leaf area. Conversely, the control/untreated plants exhibited the maximum number of leaves (11). This suggests that tomato cultivars having Cd tolerance may be cultivated in urban soils. However, it is imperative to emphasize the need for further research to understand the long-term effects and potential risks associated with Cd exposure. Investigating the sustained impact on plant growth, yield, as well as the accumulation of Cd in edible parts will provide crucial insights for ensuring food safety and environmental health in Cd-contaminated areas.

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